

Protocol for analysis of cell cycle using PI, 7AAD or ToPro-3 staining in flow cytometry

Uses and Rationale:

Propidium Iodide, 7-AAD and ToPro-3 all bind DNA at a particular point on the double helix. The amount of bound dye is proportional to the amount of DNA. As such, the amount of fluorescence is proportional to the amount of DNA. This is why we use these dyes in flow cytometry – to assess the DNA content (cell cycle) of cells.

Materials:

Propidium Iodide or 7-AAD or To-Pro-3
Ice cold methanol
RNase
PBS/1% BSA or 1% FCS

Method:

A. Cell Preparation:

1. Spin cells out of media – 1500RPM x 5 minutes
2. Wash once with PBS or PBS/1% BSA/FCS
3. Spin cells down at 2000 RPM for 5 minutes into pellet
4. It is best to have AT LEAST somewhere between $1.0\text{-}5.0 \times 10^5$ cells/tube and do each cell set in duplicate if possible (this is of course in an ideal world)
5. Add 1mL of ICE COLD absolute methanol –
6. It is **very important** how you add the MeOH: do this by beginning to vortex the tube on a medium vortex strength, and add the MeOH drop by drop while vortexing until 1mL has been added
7. Put tubes in fridge for at least 6 hours....they can sit there for as much as 6 months. Be sure, however, that caps are screwed on/fastened tightly so MeOH does not evaporate (if it does, your tube is a loss)

B. Staining:

1. Spin cells at 2000 RPM for 5 minutes
2. Aspirate off MeOH and blot tube mouths
3. Add 500uL staining RNase/propidium iodide solution: 20mg/mL propidium iodide
1mg/mL RNase in PBS
(If using 7AAD, the concentration should be 25ug/mL of 7AAD, in place of PI)
(If using To-Pro3, stock is 1mM in DMSO, stain at 1uM concentration in place of PI).
4. Vortex to remove from pellet and resuspend in solution
5. Put in fridge and covered in aluminum foil.
6. Make sure tubes are tightly capped.
7. Cells can sit and be analyzed from 30 minutes to 3 days.

C. Flow cytometry:

1. Analyze on linear with FL2-W versus FL2-A for doublet discrimination, be sure to compensate out of FL3.

Important technical notes:

General note before beginning: Stain cells for extracellular markers before adding MeOH...and if the cells have GRP/RFP/YFP or any other fluorescent protein, ask for specific instructions....it can be done, but not with this protocol, as the fluorescent protein will leak out of the cell when fixed with MeOH.